Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

10/069353

Applicant's or agent's file reference LEA33955-WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP00/08013	International filing date (*day/month/year*) 17 August 2000 (17.08.00)	Priority date (*day/month/year*) 27 August 1999 (27.08.99)
International Patent Classification (IPC) or national classification and IPC C12N 15/00		
Applicant BAYER AKTIENGESELLSCHAFT		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22 January 2001 (22.01.01)	19 November 2001 (19.11.2001)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP00/08013

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:

pages 1-37, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the claims:

pages 1-72, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the drawings:

pages 1/7-7/7, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the sequence listing part of the description:

pages 1-330, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
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III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 10,12,34-62,70-72 (all complete); 1-9,11,13-33,63-69 (all partly)

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 10,12,34-62,70-72 (all complete) ; 1-9,11,13-33,63-69 (all partly)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP 00/08013

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

1. The international preliminary examination covers those claims for which an international search report has been established, i.e. Claims 1-9, 11, 13-33 and 63-69 (all in part, that is, insofar as they concern SEQ ID NO: 7 or SEQ ID NO: 8).

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: III.

1. Claims 10, 12, 34-62, 70-72 (all in full), 1-9, 11, 13-33, 63-69 (all in part, i.e. insofar as they do not concern SEQ ID NO: 7 or SEQ ID NO:8) concern inventions for which no international search report has been established. Therefore no international preliminary examination has been carried out for these claims either (PCT Rule 66.1(e)).

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

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V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	14-26, 30-33, 63-65, 67-69	**YES**
	Claims	1-9, 11, 13, 27-29, 66	**NO**
Inventive step (IS)	Claims		**YES**
	Claims	14-26, 30-33, 63-65, 67-69	**NO**
Industrial applicability (IA)	Claims	1-9, 11, 13-33, 63-69	**YES**
	Claims		**NO**

2. Citations and explanations

1. Reference is made to the following documents; the numbering corresponds to the order in which they are listed in the international search report:

D1: KIRST, H.A. ET AL., 1992, BAKER, D.R., FENYES, J.G. AND STEFFENS J.J., AMERICAN CHEMICAL SOCIETY SYMPOSIUM SERIES NR. 504: SYNTHESIS AND CHEMISTRY OF AGROCHEMICALS III. WASHINGTON DC

D2: MATSUSHIMA P. ET AL., GENE, NL, ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, Vol. 146, No. 1, 1994, pages 39-45

D3: HOPWOOD D. A., CHEMICAL REVIEWS, US, AMERICAN CHEMICAL SOCIETY. EASTON, Vol. 97, No. 7, November 1997 (1997-11), pages 2465-2497

D4: BALTZ R. H. ET AL., TRENDS IN BIOTECHNOLOGY, GB, ELSEVIER PUBLICATIONS, CAMBRIDGE, Vol. 14, No. 7, 1 July 1996 (1996-07-01), pages 245-250

D5: WO-A-99/46387.

2. The present application does not meet the requirement of PCT Article 33(2) since the subject matter of Claims 1-9, 11, 13, 27-29 and 66 is not novel, that is, it is anticipated by the prior art as defined in the Regulations (PCT Rule 64.1 to

64.3).

The present application concerns nucleic acids which code for the enzyme activities of spinosyn biosynthesis. Spinosyns are a group of macrolidic compounds (so-called A83543 complex) which are formed from the actinomycete *Saccharopolyspora spinosa* (*S. spinosa*). The structure of the spinosyns and the fact that the spinosyns are biosynthesized via a polyketide biosynthesis pathway in which multifunctional enzymes, so-called polyketide synthases (PKSs), participate are known in the prior art (see D1). SEQ ID NO:7 (nucleotide position 828 to 1 of SEQ ID NO:4) codes for a methyltransferase (SEQ ID NO: 8, 275 amino acids).

2.1 D2 (abstract; page 40, right-hand column, paragraph 1; Figures 3 and 4) discloses the construction of a cosmid gene bank of *S. spinosa* in pOJ436 and a method of transferring cosmids containing *S. spinosa* DNA from *E. coli* to *S. spinosa*. According to D2, conjugation of *E. coli* and recombination in the chromosomal DNA of *S. spinosa* could be used to clone genes of *S. spinosa* and for complementation analyses. DNA analyses of five mutants with blocked A83543 production showed that they lacked a 400 kB SpeI fragment which was also absent from exconjugates having defective A83543 production. According to D2, these findings indicate that genes which participate in the biosynthesis or regulation of A83543 production are localised on this 400 kB SpeI fragment; this is subsequently confirmed (see D5, page 13, lines 20-32, Figure 2; only cited as technical evidence). D2 also discloses the isolated 400 kB SpeI fragment (Figures 3 and 4).

Claims 1-9 and 11 are currently so broad that they include chromosomal DNA of *S. spinosa* and the 400 kB SpeI fragment which likewise have at least one region coding for an enzyme activity that participates in spinosyn biosynthesis. A known nucleic acid (chromosomal DNA of *S. spinosa* and 400 kB SpeI fragment) is not rendered novel by the specifying of an inherent technical feature (nucleic acid sequence, SEQ ID NO: 7). *S. spinosa* is currently covered by the term "host cell" in Claims 27-29.

Thus D2 anticipates the subject matter of Claims 1-9, 11, 13, 27-29 and 31-33.

2.2 Since *S. spinosa* is covered by the term "host cell" in Claims 27-29 and D1 discloses the extraction of A83543 (= spinosyn) from the culture medium of *S. spinosa*, D1 is prejudicial to the novelty of Claim 66.

2.3 The subject matter of Claims 14-26, 30-33, 63-65 and 67-69 appears to be novel in light of the available prior art.

3. The present application does not meet the requirement of PCT Article 33(3) since the subject matter of Claims 14-26, 30-33, 63-65 and 67-69 does not involve an inventive step, that is, it can be considered obvious to a person skilled in the art according to the prior art as defined in the Regulations (PCT Rule 65.1 and 65.2).

3.1 Claim 14 is directed to a DNA construct comprising a nucleic acid which has at least one region coding for an enzyme activity (i.e. SEQ ID NO: 7) which participates in spinosyn biosynthesis and at least one heterologous promoter.

D2, which is considered the closest prior art, discloses (cf. 2.1 above) the fact that genes which participate in the biosynthesis or regulation of A83543 production in *S. spinosa* are localized on a 400 kB SpeI fragment. The subject matter of Claim 14 differs from the prior art in that it is directed to a DNA construct which comprises a nucleic acid that codes for an enzyme activity (i.e. SEQ ID NO: 7).

The object of the present invention can thus be considered that of preparing those nucleic acids which code for the enzyme activities of spinosyn biosynthesis (i.e. SEQ ID NO: 7).

For the following reasons, the way of achieving this object proposed in Claim 14 of the present application cannot be considered inventive (PCT Article 33(3)):

D3 (entire document) provides an overview of the cloning of PKSs and discloses that the PKSs participating in macrolide biosynthesis in actinomycetes consist of modules and that the corresponding biosynthesis genes occur in a single cluster. D3 also mentions the possibility of cloning the biosynthesis genes by means of complementation and presents numerous examples of cloned actinomycete PKSs.

D2 prepares a method which can be used specifically for cloning genes of *S. spinosa* and for complementation analyses in *S. spinosa*. D2 further discloses that genes which participate in the biosynthesis or regulation of A83543 production in *S. spinosa* are localized on a 400 kB SpeI fragment.

Therefore the Examining Authority is of the opinion that the preparation of the nucleic acids which code for the enzyme activities of spinosyn biosynthesis (i.e. SEQ ID NO: 7) was obvious to a person skilled in the art in view of the teaching of D2 combined with D3. A person skilled in the art would also have a realistic expectation of success since the identification and characterization of the claimed specific sequences have no other significance for a person skilled in the art than the routine performance of experiments.

3.2 Claims 15-26, 30-33, 63-65 and 67-69 concern embodiments which are known to a person skilled in the art. They would be considered inventive only if they involved a novel and inventive nucleic acid. This is not the case for Claims 15-26, 30-33, 63-65 and 67-69. Therefore, in light of the available prior art (D1-D4), the subject matter of these claims is considered non-inventive.

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
WO 99 46387	16 September 1999 (16.09.1999)	16 February 1999 (16.02.1999)	09 March 1998 (09.03.1998)

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: VI.

Assuming that the sequences of SEQ ID NO: 7 in the first priority document (DE19991040596, 27 August 1999) and in the present application are identical (this has not been verified), the claimed priority date can be recognized for the relevant parts of the present application and WO-A-99/46387 is not considered prior art under PCT Article 33(2) and (3) (PCT Rule 64.3).

WO-A-99/46387 discloses the sequence of spnF (SEQ ID NO: 1, 20168-20995), which is identical to SEQ ID NO:7 of the present application.